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(54) Title: DIETETIC AND/OR PHARMACEUTICAL COMPOSITIONS FOR HUMAN AND/OR ANIMAL USE BASED ON PROBIOTIC MICROBIAL PREPARATIONS

(57) Abstract: The present invention relates to dietetic and/or pharmaceutical compositions for human and/or animal use, and general foodstuffs, based on microbial cultures consisting of autochthonous and allochthonous species with respect to human beings and animals, selected from species of lactic bacteria, propionibacteria, yeasts and/or molds. They have an equilibrating action of the intestinal flora of the host (human being or animal), as well as having various beneficial/probiotic effects towards the host organism.

5

DIETETIC AND/OR PHARMACEUTICAL COMPOSITIONS FOR HUMAN AND/OR ANIMAL USE BASED ON PROBIOTIC MICROBIAL PREPARATIONS

The present invention relates to dietetic and/or pharmaceutical compositions for human and/or animal use based on probiotic microbial preparations.

In particular, the present invention relates to dietetic and/or pharmaceutical compositions for human and/or animal use and foodstuffs in general, based on microbial cultures consisting of autochthonous and allochthonous species with respect to human beings and animals, selected from the lactic bacterial species *Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei* subsp. *casei, Lactobacillus casei* subsp. *rhamnosus, Lactobacillus zeae, Lactobacillus salivarius, Lactobacillus lactis, Lactobacillus helveticus, Lactobacillus reuteri, Lactobacillus amylovorus, Lactobacillus crispatus, Lactobacillus curvatus, Lactobacillus delbrueckii* subsp. *delbrueckii, Lactobacillus* delbrueckii and all its subspe-

cies, Lactobacillus *gasseri*, *Lactobacillus johnsonii*, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, optionally associated with *Streptococcus thermophilus*; *Lactobacillus fermentum*, *Lactobacillus brevis*, *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Leuconostoc* spp.; *Entercoccus faecium*, *Pediococcus pentosaceus*, *Pediococcus acidilactici*; Bifidobacteria such as *Bifidobacterium Longum*, *Bifidobacterium Breve*, *Bifidobacterium bifidum*, *Bifidobacterium infantis*, *Bifidobacterium lactis*; and/or propionibacterial species, yeast species and/or mold species; the above species being live and vital and/or devitalized, and said species being present in microbial cultures in a dried concentrated form with a concentration ranging from 10^6 ufc/g to 10^{11} ufc/g.

The above-mentioned probiotic micro-organisms shall be indicated hereafter with the term "pr micro-organisms".

The compositions, object of the present invention, have an equilibrating action of the intestinal flora of the host (human being and animal), in addition to producing various beneficial/probiotic effects towards the organism which vary according to the destination target, such as children, adults, elderly people, expectant women, persons with various kinds of deficiencies or gas-

tro-intestinal disturbances (dismicrobisms) or with acute or chronic diseases, for example of the vaginal or urological type.

These components, with a reciprocal association, act according to a biological and synergic succession on an intestinal level.

It is also known that bacteriophages and bacteria form a more or less indissoluble pair to the extent that it can be declared that there are no micro-organisms without bacteria. The development of bacteriophages is generally considered as being a production problem, but it can also be observed in intestinal flora after the ingestion of microbial preparations which exert a probiotic effect. In this case, the problem must be solved in the product formulation phase so as to preserve the positive effects of the composition.

It has been found that the most effective solution consists in the differentiation of the species and availability, for each of these, of numerous strains with a different sensitivity to bacteriophages so that all the species are always present in the intestines.

If, on the contrary, the product only consists of a single strain, it is not possible to guarantee the presence of all the species in the case of lysis of this strain and the beneficial effects obtainable with their

ingestion will be lost.

The positive effects of a bacterial culture consisting of a single strain of a certain species are consequently rather limited as it can be easily attacked by its specific bacteriophages.

Another factor which can inhibit the development of bacteriophages is the administration of preparations for oral bacterium therapy for quite a limited period of time in order to reduce the possibility of the development of bacteriophages.

When preparations for oral bacterium-therapy are administered for very prolonged periods of time to a large number of individuals, as is the case for example in hospitals, the risk of phagic infection is extremely high. The problem can be solved by substituting the strains used with other strains definitely resistant to those specific bacteriophages.

Bacteriophages are an extremely important biological reality, even more widely diffused than micro-organisms themselves. Cases of diarrhea not due to the action of pathogenous germs can be correlated to the phagic attack of some normal constituents of intestinal flora. It should be pointed out that phages are harmless for human beings even though they may interfere with the intestinal flora.

The continuous administration of bacteria of the same strain, with a probiotic action, leads to the development of specific bacteriophages which destroy said bacterial strain, thus annulling its probiotic prophylaxis. A knowledge of bacteriophages can be of help in the preparation and use of cultures adopted in oral bacterium-therapy.

In order to obtain a effective protection with respect to intestinal disturbances, the problem of bacteriophages has been considered and solved with the composition according to the present invention.

An object of the present invention therefore also relates to a composition which comprises different strains of the same species having a different sensitivity to bacteriophages (lysogeny and lysotypy) but with the same biological and probiotic properties.

The composition according to the present invention can also comprise at least one of the following components: other micro-organisms, enzymes, mineral salts, vitamins, prebiotics, natural fibres, phyto-derivatives, antioxidants, fermented milk, paps, feeds.

The live and vital and/or devitalized yeasts of the compositions, object of the present invention, are yeasts with a low fermentative capacity for probiotic use, rich in essential amino acids. In particular, the yeast can be

Saccharomyces cerevisiae or *Saccharomyces boulardii*.

In the composition according to the present invention, at least one of the pr micro-organisms is preferably present in a concentration lower than 10^9 ufc/g.

In particular, a dietetic and/or pharmaceutical composition according to the present invention comprises *Streptococcus thermophilus*, Bifidobacteria such as *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium lactis*, *Bifidobacterium bifidum*, *Lactobacillus acidophilus* in a concentration ranging from 10^9 to 10^{11} ufc/g, *Lactobacillus plantarum*, *Lactobacillus casei* subsp. *casei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Enterococcus faecium* in a concentration ranging from 10^6 to 10^9 ufc/g.

In particular, *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* are developed in symbiosis (or protocooperation).

The natural enzymes used essentially consist of a mixture made up of β-glucanase and xylanase produced by micro-organisms of the Thricoderma type.

The mixture comprising these natural enzymes is particularly useful for optimizing the digestive catalase of hydrosoluble polysaccharides (NSP). This mixture develops a synergic action on the various NSP contained in wheat, barley, oats, rye and triticum, and is defined by its

specific efficacy on the various substrates, the analytical characteristics of the single components, the product properties studied for commercial use in association with a pool of pr micro-organisms in human and animal nutrition.

The natural fibres, possibly present in the composition according to the present invention, are selected from fibres of acacia, oats, apples, inulin, psyllium, microcrystalline cellulose (which act as prebiotics).

The composition according to the present invention comprising pr micro-organisms and prebiotics, such as natural fibres, is of particular interest.

The fibres represent a nutritional substrate for the pr micro-organisms. The metabolic activity of pr micro-organisms on the fibres (prebiotic) makes them easier to digest and causes the free release of substances useful for the nutrition of the organism (human or animal), of the pr micro-organisms and autochthonous intestinal flora.

The phyto-derivatives are preferably selected from extracts from Eleuterococcus and green tea.

The antioxidants are preferably natural antioxidants and can be selected from oleuropein (from extravirgin olive oil), lycopene (from tomatoes), bioflavonoids (from citrus fruit), phenol components (from red grapes).

The vitamins and mineral salts are selected from vitamin A, B1, B2, B6, B12, niacin, C, D3, E, folic acid, calcium, phosphorous, magnesium, iron, zinc.

A further object of the present invention therefore relates to the use of said dietetic and/or pharmaceutical compositions as integrators and/or dietetic-therapeutic products for human and/or animal nutrition.

In particular, an object of the present invention also relates to the use of said dietetic and/or pharmaceutical compositions for preparing integrators, dietetic-therapeutic food products, food, drinks and/or feeds for human and/or animal nutrition.

The food can consist of milk, cheese, paps, homogenized products (based on meat, milk, cheese, fruit, vegetables), dietetic food products destined for diabetics such as jams, chocolate, sweeteners other than sucrose, or animal feeds.

The milk can be fermented or non-fermented milk, with the direct inoculum of suitable pr micro-organisms in a concentrated dried form.

The pr micro-organisms in a concentrated dried form inoculated into milk are suitable for the processing of 1000 or more liters of milk without any intermediate passage with the presence on the finished product of the probiotics identified.

Therapeutic dietetic cheese can be obtained by the addition of suitable pr micro-organisms in a concentrated dried form in a certain processing phase of the cheese in order to guarantee, in a certain gram-weight of cheese, the supply of the dose of pr micro-organisms necessary for the organism.

The drinks can be instantaneous drinks or water containing the compositions according to the present invention.

The present patent application also relates to integrators, food, dietetic-therapeutic food products, drinks and/or feeds for human and/or animal nutrition, characterized in that they contain a dietetic and/or pharmaceutical composition according to the present invention.

The use of the compositions according to the present invention for human nutrition has an equilibrating action on the intestinal flora of the host (human being and animal), in addition to producing various beneficial/probiotic effects towards the organism which depends on the destination target (children, adults, elderly people, expectant women, people with various kinds of deficiencies or gastro-intestinal disturbances i.e. dismicrobisms, or with acute or chronic diseases, for example of the vaginal or urological type). This use also regulates intestinal functioning, improves constipation, hemor-

rhoids and intestinal irritation; it reduces the absorption of sugar and cholesterol.

The use of the compositions according to the present invention in zootechnics allows growth promotion, the control of enteric pathologies of a bacterial origin, an improvement in the digestive efficiency (I.C.A.) of animals for breeding, the food conversion index and an improvement in the quality of the end-product (meat or eggs, in the case of fowl), thus solving problems linked to antibiotic residues.

In addition to this, the use of the compositions according to the present invention results in a better consistency of feces, a reduction in enteric pathologies of a bacterial origin (colibacillosis), an improvement in the consistency of the egg-shells produced, an improvement in the egg-laying itself, a reduction in therapeutic treatment and an improved immunitary response.

In particular, as far as human nutrition is concerned, the compositions according to the present invention, containing suitable live and vital and/or devitalized pr micro-organisms in concentrated dried form, can be used as a dietetic and/or therapeutic product in powder form as tablets, capsules or sachets both for pharmaceutical use and as food integrators, optionally combined with natural principles.

Pr micro-organisms can, in fact, interact with nutritional principles of a natural origin such as fibres of acacia, oats, apples, Psyllium - which already have health-giving properties and whose reduced food contribution can be correlated with various pathologies, such as constipation, obeseness, cardiovascular diseases, diabetes.

These substances, also classified as prebiotics, interact with pr micro-organisms (such as lactic bacteria and bifidobacteria) as they act as a substrate on which the pr micro-organisms themselves develop thus creating a synergic effect. The symbiotic combination between pr micro-organisms and prebiotic fibres, such as inulin, favours intestinal functioning, improves constipation, hemorrhoids and intestinal irritation; it reduces the absorption of sugar and cholesterol.

Another extremely interesting combination is that between pr micro-organisms and antioxidants of a natural origin, such as: bioflavonoids, anthocyanins, lycopene, orthophenols, extracts from citrus fruit, red grapes, tomatoes, olive oil, respectively. These antioxidants are not only effective in fighting AOR (Active Oxygen Radicals), but they are also capable of protecting the integrity and functionality of microflora on an intestinal level, guaranteeing a greater beneficial effect on human

beings.

Pr micro-organisms can also be "coupled" with vitamins and minerals, especially in cases where a lack of these is likely. Deficiencies in specific trace elements are now associated with chronic health problems: typical examples are fluorine and dental decay, chromium and tolerance to glucose, copper and hypercholesterolemia, zinc and the immunitary system.

The association of vitamins and minerals with pr micro-organisms improves the absorption, i.e. bioavailability, of nutritional principles, a factor which is often neglected in evaluating the composition of integrators and/or the necessity for their use. A second important aspect is linked to the action synergy, for example between zinc and pr micro-organisms which are both implied in the modulation of the immunitary function.

This synergy also develops between various species of vegetable substances, phyto-derivatives (extracts from Eleuterococcus and green tea), known for their tonic, adaptogenous, antistress, immuno-potentializing activity, but at the same time a hypocholesterolemizing, antioxidant activity, for controlling glycemia, modulating the ecosanoid cascade, with safe health effects.

The combination with pr micro-organisms favours the effect of phyto-estrogens as they increase the bio-

availability and absorption of these vegetable principles, in addition to normalizing the equilibrium of intestinal flora altered in situations of stress.

Pr micro-organisms in association can also be used for enriching dietetic food products destined for children and adults, for example in homogenized products (based on meat, milk, cheese, fruit, vegetables, etc.) or in products destined for diabetics (jams, chocolate, sweeteners other than sucrose), etc.

The pr micro-organisms can be mixed with the above food preparations, with fermented milk and with food products in general, in different proportions.

The addition to food of pr micro-organisms in concentrated dried form is justified in that these products have equilibrating properties of intestinal flora and stimulate the immunological properties and natural defence system of the organism in addition to those against tumours.

In animal nutrition, on the other hand, the compositions according to the present invention, containing live and vital and/or devitalized pr micro-organisms, in a dried concentrated form, can be used for the following purposes.

The biological processes linked to microbial life present with respect to the enteron are extremely impor-

tant for animals, as they can influence both the digestive processes and also the absorption processes of the nutritional principles. In this respect, two main types of micro-organisms can be distinguished on an intestinal level: fermentation micro-organisms which produce, starting from glucosides, various short-chain fatty acids and putrefaction micro-organisms which degrade the amino acids producing biogenic amines. Under normal physiological conditions, the putrefaction processes are controlled by fermentative processes, whereas when there is an enteric pathology, bacterial strains with a high putrefactive action, above all *Escherichia coli*, are predominant.

In this particular context, resort is made to the use of selected cultures of pr micro-organisms which form the typical and most effective antagonist of putrefactive flora. The antagonist action is in fact carried out by means of a "barrier effect", exerted with adhesion to the intestinal epithelium, and an acidifying activity which makes the enteric environment unsuitable for the development of pathogenous flora. Suitable specifically combined pr micro-organisms allow the development of a beneficial microflora to be activated on an intestinal level. This is an innovative technology which is extremely effective and completely without side-effects, for controlling enteritis of a bacterial origin and for improving the di-

gestion of breeding animals, the food conversion index and problems linked to residues in meat or eggs (in the case of fowl). In particular, the following selection criteria are adopted for selecting the strains which form these cultures:

- adhesion capacity to the epithelium of the enteron;
- resistance to gastric acidity;
- development and production rate of lactic acid at the level of the enteron;
- processing of the enzymes useful for food degradation.

100,000,000 quintals of feed form the basic datum for evaluating the potential market of selected cultures of pr micro-organisms used as growth promoters and as active principles for the prophylaxis of enteric diseases. In both cases, the advantages in terms of quality improvement (in particular of meat, reduction in the use of antibiotics and consequently the elimination of residues) which products based on pr micro-organisms offer, are important.

In addition to a greater control of enteric pathologies of a bacterial origin and an improvement in the digestive efficiency (I.C.A.), the application of lactic bacteria shows:

- an improved consistency of feces;

- a reduction in enteric pathologies of a bacterial origin (colibacillosis);
- an improvement in the consistency of the egg-shells produced;
- an improvement in the laying;
- a reduction in therapeutic treatment;
- an improvement in the immunitary response.

In particular, technology development in animal nutrition is increasingly emphasizing the problem of the use of antibiotics for auxinic purposes in feeds.

The pros and cons, advantages and disadvantages, use and abuse of these molecules have often been the object of discussion between scientists, which frequently involves the public. In England, the "Swam Institute" published a report in 1969 in which the use of antibiotics for zootechnical productions was connected to the risk of allergic or toxic reactions, and to the danger of the formation of antibiotic-resistant microbial strains, with cases of a wide diffusion of resistance to said antibiotics from animals to human beings. When antibiotics are absorbed by animals, in fact, they can be found in the form of residues in meat.

There is also the problem relating to possible intolerance on the part of those working in feed producers and of zootechnical operators, who can come into contact

with the various active principles.

The identification and use of natural auxinic factors which do not produce negative effects such as those mentioned above, is therefore of great interest.

The use of gastrointestinal flora "modulators", such as yeasts, lactic bacteria and bifidobacteria, is becoming more and more well known in the preparation of food destined for animal nutrition of the concentrated type or in the form of fodder.

Limitations in the use of these products derive from the current legislations in force which only allow the use of some species of pr micro-organisms (lactic bacteria and yeasts), at very low concentrations, which often jeopardize the efficacy in zootechnical applications.

Detailed studies in the selection and preparation of new species of pr micro-organisms suitable for use in various zootechnical species, are consequently extremely important.

Another differentiation to be taken into consideration as a selection criterion of these products is the different action mechanism according to the animal species, distinguishing between monogastric species and those with a polygastric digestive system.

The formulations of the composition according to the present invention are polyvalent and therefore play an

important role in the preventive treatment of gastric and intestinal diseases.

These preparations contain not only pathogen agent antagonists, but also compounds which produce biologically active substances for regulating the metabolic processes in animals and raising their resistance to infections.

Said compositions consist of a mixture of pr microorganisms (for example, lactic bacteria, propionibacteria and yeasts) in a concentrated and dried form.

Lactic bacteria are in fact antagonists of many pathogenous varieties and are vitamin producers, raising the resistance of the organism to illnesses. Propionibacteria (which form part of the intestinal microflora of ruminants) produce propionic acid (important for the regular growth of calves), acetic acid (essential for the synthesis of milk fat, during lactation) and B12 vitamins.

For yeasts, biomasses have been selected and produced, which due to their biochemical characteristics, increase their probiotic properties and are functional for zootechnical use, also identifying the specificity for the different animal species and various productions. This solves the present situation whereby yeasts destined for zootechnical use consist, in the best of cases, of

surpluses of yeast production for bread-making, with the selection and production of yeasts which therefore have:

- a high adaptability under the conditions present in the digestive system of animals;
- a high content of particular cellular elements (amino acids, vitamins, etc.).

Yeasts favour a series of important biochemical reactions in the rumen, which improve the fermentative activity, allowing the contemporaneous presence, in milk cows, of the three volatile fatty acids precursors of milk components (fat, casein and lactose), in the highest possible concentrations.

In the zootechnical field, in particular as far as cattle breeding is concerned, it has been demonstrated that autochthonous microflora affects the animal's health as it can participate in the digestion and metabolism of the nutritive substances, supplying energy, amino acids and sugar which could otherwise not be available to the host.

An appropriate equilibrium between the autochthonous micro-organisms normally present in the intestines or rumen, ensures, among other things, the mutual beneficial relationship between host and microflora and consequently the health of the animal.

As a result, the treatment of animals with the poly-

valent compositions according to the present invention, consisting of a mixture of pr micro-organisms (for example lactic bacteria, propionibacteria and yeasts), creates not only an antagonistic action against pathogenous agents, but also the production of biologically active substances which regulate the metabolic processes of animals and raise their resistance to infections.

To actuate similar treatment in intensive breeding it is necessary however to have cultures which are highly concentrated in cells and are dried, to facilitate transportation and use.

In conclusion, the use of the compositions according to the present invention containing pr micro-organisms in a concentrated and dried form to be used as food integrators for cattle leads to:

- an improvement in the state of health of animals with a consequent increase in meat yield;
- a reduction in the use of antibiotics in feeding breeding cattle, with indirect effects for consumption due to a greater hygienic reliability of the meat;
- positive ecological consequences on the waste water disposal of farms together with the advantages already mentioned and deriving from the concentrated and dried form (multiple and simple use and easy transportation).

According to research effected on fowl species

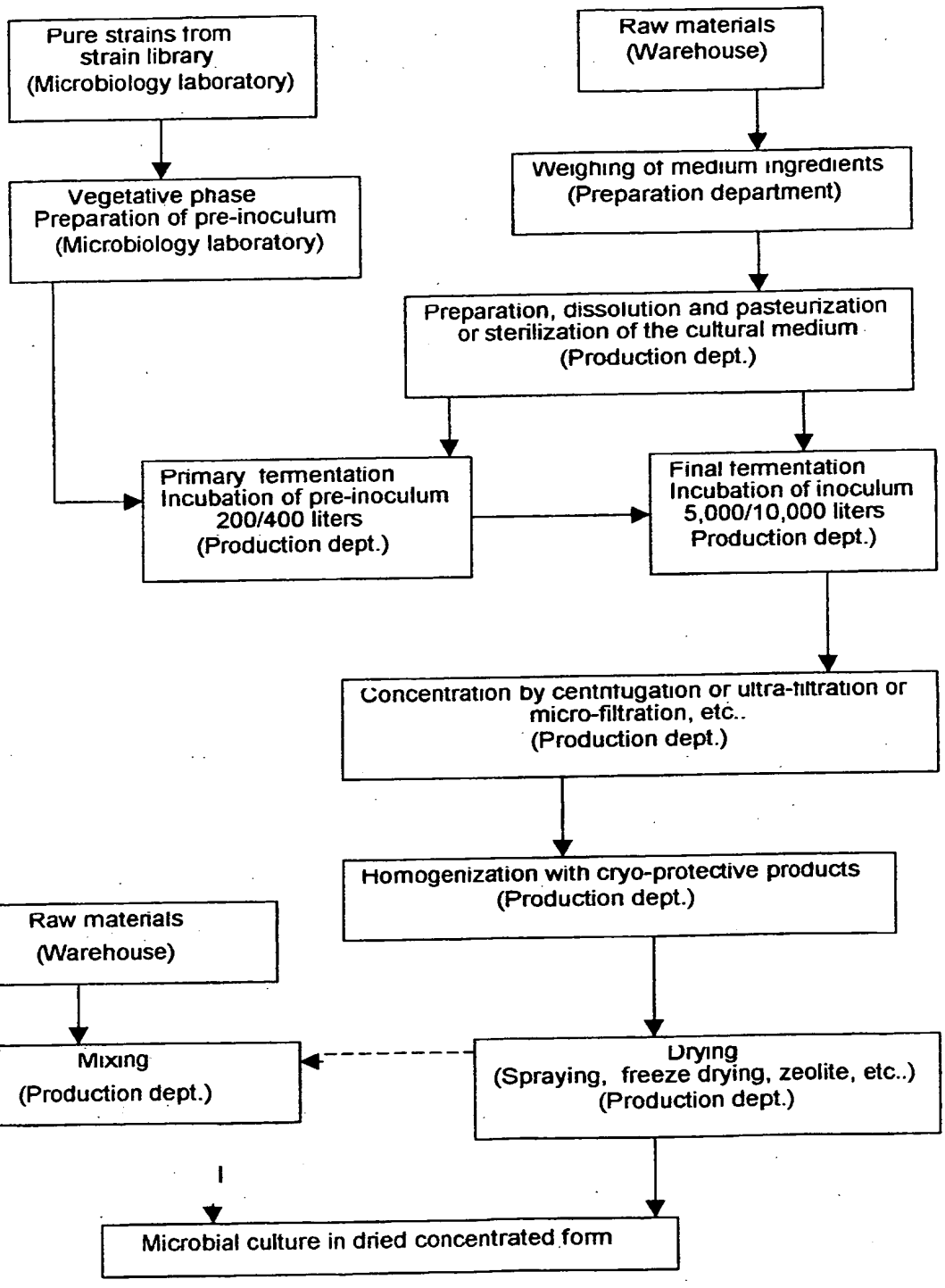
(hens, broilers, turkeys) it has been found that the administration of specific cultures of pr micro-organisms, is extremely effective from the very first day of the animal's life.

The development of a totally beneficial intestinal flora and consequently the start of the productive cycle under optimal functioning conditions of the digestive system, has, in fact, been observed.

The use of pr micro-organisms in animal breeding is extremely practical as it can be effected both via the food (feed) and also via the drinking water, care being taken not to subject the pr micro-organisms to temperatures higher than 65-70°C, so as not to jeopardize their vitality.

The pr micro-organisms selected for the compositions object of the present patent application have different nutritional demands and require the application of a wide variety of fermentative parameters (growth temperatures, pH conditions, fermentation times, drying curves, etc.) for which a general production scheme is provided below (Scheme 1)

SCHEME 1 – Process diagram



For illustrative purposes only, the manufacturing process is described below of one of the components of the mixtures of pr micro-organisms such as the species *Streptococcus thermophilus*.

The manufacturing process comprises a vegetative phase in a flask, starting from collection strains (in a laboratory), a production phase including primary and final fermentation and the actual production phase (concentration, homogenization, drying).

Vegetative phase

Preparation of the pre-inoculum

Different strains of the same species can be used for the manufacturing of the species *S. thermophilus*.

For the preparation of the pre-inoculum (vegetative phase) the contents of the various phials containing the collection strains (1 per strain) are dissolved separately with 1.5 ml of a solution of peptone and sterile salt. The suspensions contained in the phials are mixed and inoculated in equal quantities into 10 ml test-tubes of sterile skimmed milk or dried milk re-formed at 10% in water. They are incubated at 37°C until the milk coagulates or until the logarithmic growth phase of the culture thus obtained (first culture).

The first culture is transferred to a 250 ml flask of sterile skimmed milk. It is incubated at 42°C until

coagulation (second culture) and, if necessary, it can be conserved at 5°C for 3 days.

The contents of the second culture are transferred (in a ratio of 2% = 20 ml/l) into a flask containing 4 l (or 8 l in relation to the expected fermentation volume) of a substrate of re-formed sterile skimmed milk in a ratio of 10% and containing 1% of yeast extract and incubated at 37°C for about 2 hours (until coagulation). The flask is then left to cool in a refrigerator (4°C) until use. The phase contrast microscopic morphological control, the growth and acidification curve control and the control of the contaminating germs and acidity developed are then contemporaneously effected on the third culture (laboratory preinoculum).

1st production phase

Primary fermentation

This phase is carried out in a 200/400 litre fermentor using 200 l (or 400 l in relation to the expected fermentation volume) of dried skimmed milk re-formed at 8% (containing 0.1% of Antifoam additive), as culture medium. The components of the medium were previously weighed and dissolved, using an automatic plant. Pasteurization of the culture medium is then effected, under slow stirring, at 90°C for 30 minutes. The medium is cooled to 42-44°C and is sterilely inoculated with the

contents of the flask (preinoculum, 4 or 81) of the vegetative phase. The fermentation is continued for 2-3 hours, after which rapid cooling is initiated to 4-8°C with icy water.

The culture is maintained at 5°C until the end of the quality control.

Final fermentation

Preparation of the culture medium

Culture medium 5000 l (or 10000 l).

A 5000 l fermentor is used (in the case of 10000 l the quantities are doubled), previously washed and flushed with a CIP cycle. 4400 liters of water are charged, and the fermentor is heated to 60°C.

The following products are weighed:

- Lactose 80 Kg
- Yeast extract 25 Kg
- Dextrose 150 Kg
- Ammonium sulfate 15 Kg
- Monobasic potassium phosphate 7.5 Kg
- Manganese sulfate 0.5 Kg
- Antifoam additive 0.5 Kg

The components of the medium are dissolved using an automatic plant. Pasteurization is then effected, under slow stirring, at 80°C for 30 minutes.

Inoculation and incubation

The culture medium mass is cooled to 40-42°C; it is sterilely inoculated with 200 liters of the primary fermentation culture and is thermostat-regulated; it is kept under stirring and incubated for 3-4 hours approximately, controlling the pH which is continuously adjusted with NaOH at 30% to maintain a value of 6.1-6.2. The regulation is effected continuously, under stirring.

Various samplings are taken for the different controls and rapid cooling to 5°C with icy water, is initiated. The operations are carried out so as to complete the fermentation within the same day, enabling the microbiological purity control (e.g. absence of coliforms) to be effected before the operations of the following day.

2nd production phase

Concentration of the cellular mass (e.g. by centrifugation)

Preparation of the centrifuges

The Westphalia centrifuges (or ALFA LAVAL) are washed and flushed using a CIP plant with NaOH and nitric acid, washed with hot water and pre-cooled (hollow cavity) with icy water to 5°C.

The culture to be concentrated is directly transferred, by means of a lobe pump, to the centrifuges, using a closed circuit plant, flushed in CIP, maintaining a flow-rate of 1000 l/hour (2500 l/hour for ALFA LAVAL).

The first three discharges of concentrate are eliminated and the whole of the remaining quantity is directly transferred, still in line, to the homogenizer.

Homogenization of the concentrate with cryoprotective products

The concentrate collected is added with a cryoprotective product (in a ratio of 16% with respect to the weight of the concentrate collected) and homogenized. The cryoprotective product is thus composed: (dose per 300 Kg of concentrate):

- Dried skimmed milk	4.0 Kg
- F.U. Lactose	12.8 Kg
- Sucrose	4.0 Kg
- Yeast extract	3.2 Kg
- Water	24.0 l

(the cryoprotective product is previously prepared and pasteurized at 80°C), the pH is corrected to 6.3-6.5 with NaOH at 30%, samplings are taken for the controls and it is transferred to a vacuum freeze drier.

At the end of the operations, the centrifuge is immediately flushed with nitric acid - soda in CIP, and is rinsed, first with rinsing water and then with hot water. The homogenizer is immediately flushed with nitric acid - soda and water at 90°C.

Drying (e.g. by vacuum freeze drying)

All the vacuum freeze drying accessories must be flushed, before use, in compliance with the specific procedures whereas the chambers and fixed parts are decontaminated with vapour and/or a solution of sodium hypochlorite or another suitable decontaminant.

The concentrate is directly distributed by means of a pump to the vacuum freeze drying basins, stratifying about 1.5 cm per basin (also in relation to the actual quantity of material obtained). The vacuum freeze drying process is activated according to the automatic program of the lyostat; the vacuum freeze drying is considered as being terminated (normally after about 36-40 hours) when all the temperature registration curves of the product are stable for at least 8 hours and the chamber isolation test does not reveal vacuum drops higher than 10% (less than 100 microbar).

The basins are extracted from the lyostat, the product is immediately discharged and the lyophilized product is collected in double polyethylene bags. The product is ginned on an oscillating blade granulator previously washed and equipped with an inox grid of 2.77 x 1 mm and collected in double polyethylene bags or in steel drums. Each drum or bag is sampled for control and immediately placed in a quarantine cell. At the end of the drying process, the yield % to dried product obtained is calcu-

lated, with respect to the damp concentrate.

Final mixing (standardization and stabilization)

The product destined for the use listed above can consist of the mixture of several pr micro-organisms in a predefined cellular ratio. This predefinition obviously cannot be actuated in a hypothetical fermentation in a mixed culture but can only be effected from an appropriate mixing of the single species which takes into account the single cellular numerical charges of the single components.

Through said mixing, (in addition to the necessary "titre" standardization according to specifications) the result of a stabilization of the cellular numerical charge is reached, together with its ratios between the various species. For this purpose, suitable inert diluents are used (such as F.U. Lactose or, alternatively and for use in lactose-free specialties, maize starch, SiO_2, Mg Stearate).

The mixing is effected using a 750/1000 l biconic mixer, previously washed and flushed with a suitable decontaminating agent, by means of a validated mixing cycle. The diluent can be added in two or three successive aliquots, in relation to the relative quantities.

At the end of the mixing, the product is discharged into double polyethylene bags, and immediately sampled

for specific quality controls; the bags are immediately thermo-sealed and placed, in adequate packaging, in the quarantine area of the refrigerator at +2/+8°C "Awaiting Analysis".

Some typical schemes are provided below for the preparation of mixtures standardized and stabilized with a predetermined titre. As they are raw materials to be dosed at a *minimum vital cellular charge*, (UFC - Unit Forming Colonies), the quantities naturally vary slightly from lot to lot, whereas the diluent must, necessarily, be calculated each time according to the a.n. (as necessary) scheme.

Scheme a) (total microbial charge not less than 300 billion cells/g)

200 Kg Lot

Species	*(Minimum) UFC Quantity*
S. thermophilus	4.6×10^{16}
Bifidobacteria	2.1×10^{16}
L. acidophilus	4.4×10^{14}
L. delbrueckii subsp. *bulgaricus*	0.68×10^{14}
L. plantarum	0.50×10^{14}
L. casei	0.50×10^{14}
E. faecium	0.068×10^{14} (minimum 50 g)
F.U. Lactose a.n.	200 Kg

Scheme b) (total microbial charge not less than 100 bil-

lion cells/g)

200 Kg Lot

Species	*(Minimum) UFC Quantity*
S. thermophilus	$1.54x10^{16}$
Bifidobacteria	$0.7x10^{16}$
L. acidophilus	$1.47x10^{14}$
L. delbrueckii subsp. *bulgaricus*	$0.234x10^{14}$
L. plantarum	$0.174x10^{14}$
L. casei	$0.174x10^{14}$
E. faecium	$0.023x10^{14}$ (minimum 50 g)
F.U. Lactose a.n.	200 Kg

Scheme c) (total microbial charge not less than 70 billion cells/g - Lactose free)

200 Kg Lot

Species	*(Minimum) UFC Quantity*
S. thermophilus	$1.07x10^{16}$
Bifidobacteria	$4.9x10^{15}$
L. acidophilus	$1.03x10^{14}$
L. delbrueckii subsp. *bulgaricus*	$0.16x10^{14}$
L. plantarum	$0.11x10^{14}$
L. casei	$0.11x10^{14}$
E. faecium	$0.016x10^{14}$ (minimum 50 g)
SiO_2	2.0 Kg
Mg Stearate	1.0 Kg
F.U. Lactose a.n.	200 Kg

The quality control of the formulation after mixing is effected as described below.

Control of the differential microbial charge in the mixture.

Peptone-salt diluent: 1 g of peptone and 8.5 g of NaCl are dissolved in 1000 ml of water; the mixture is stirred carefully, heated and, if necessary, the pH is adjusted so as to obtain, after sterilization in an autoclave, a value of 7.0 at 25°C.

Method: 10 g approximately, carefully weighed, of dried concentrated culture are diluted to 100 ml (dilution 1:10) with the diluent solution.

The mixture is stirred carefully and homogenized in a Stomacher homogenizer for 1.5 minutes and the cells are left to revitalize in a thermostat at 37°C for 20 minutes. 10 ml of the first dilution (10^{-1}) are transferred to an empty sterile bottle, diluted with 90 ml of diluent (10^{-2}) and stirred carefully. The decimal dilutions are repeated in succession to 10^{-9}; the dilutions thus obtained will be used in triplicate form.

Preparation of the plates for the counting of the pr micro-organisms: Three replicates per dilution to be examined are distributed on an adequate number of 90 mm Petri plates, together with 14 ml of HHD commercial broth, containing 20 g/l of agar and 1 g/l of Tween 80.

The mixture is left to cool and dried under a horizontal flow hood. 0.1 ml of the pre-selected dilution is planted at the centre of each plate and is distributed by paletting/rotation with a 70-75 mm glass spatula until the complete absorption of the planting. The plates are incubated in anaerobiosis with a Gas-Pack system for 72 hours at 37°C

The counting is effected (referring to the different dilutions) by distinguishing the various species on the basis of the different morphologies, colouring of the colony and by means of stereoscopic microscopic control. When there are doubts, a part of the colony in question is removed with a needle and a microscopic preparation is transferred to a sample slide with a drop of sterile water and is covered with a covering slide. It is observed in phase contrast with a 40x lens and a 10x eyepiece (for Bifidobacteria a 100x immersion lens is used).

Examples of compositions based on probiotic microbial preparations for human and/or animal use.

The examples are provided for illustrative purposes only and in no way limit the scope of the present invention.

EXAMPLE 1

Homogenized products destined for children

1 g of live and vital *L. bulgaricus* and *S. thermo-*

philus (in concentrations of 1 to $5x10^9$ ufc/g), in symbiotic association, in a dried concentrated form are mixed with 50-100 grams of homogenized product.

EXAMPLE 2

Food integrators based on live and vital lactic bacteria and bifidobacteria.

These integrators contain the following live and vital pr micro-organisms:

St. thermophilus	~ 27%	equal to	$540x10^6$ ufc/g
Bifidobacteria	~ 20%		$400x10^6$ ufc/g
Lc. lactis, Lc. cremoris, Leuconostoc sp	~5.5 %		
		equal to	$110x10^6$ ufc/g
L. casei	~ 8%		$100x10^6$ ufc/g
L. helveticus	~ 5.5%		$110x10^6$ ufc/g
L. plantarum	~ 37%		$740x10^6$ ufc/g
	Total 100%	Total	$2000x10^6$ ufc/g

and also contain, according to the use and for illustrative purposes, the following compounds:

A) natural fibres (fibres of acacia, oats, apples, inulin, psyllium, microcrystalline cellulose) for regulating the intestines and weight control.

The integrator thus obtained is particularly suitable for hypocaloric diets, constipation, hemorrhoidal disturbances, for the prevention of obesity, varicose veins, intestinal irritation;

B) natural antioxidants for free radical defence, and anti-aging effect.

The antioxidants comprise oleuropein (from extravirgin olive oil), lycopene (from tomatoes), bioflavonoids (from citrus fruits), phenol components (from red grapes). The integrator thus obtained is suitable for people subject to oxidative stress deriving from unbalanced nutrition, a disorderly life-style, cigarette smoke and pollution, it is also suitable for the prevention of degenerative and cardiovascular diseases and against deterioration of the cellular functions, including aging;

C) vitamins and mineral salts for the re-equilibrium of the intestinal flora with a supply of nutritional substances.

The nutritional substances comprise vitamins A, B1, B2, B6, B12, niacin, C, D3, E, folic acid, calcium, phosphorous, magnesium, iron, zinc.

This combination is suitable for people following hypocaloric diets and do-it-yourself dieters, those who carry out moderate or intense sporting activities, elderly people and inappetent children, adolescents in the development phase, expectant women and during breastfeeding, smokers, after the administration of antibiotics and as a preventive treatment for osteoporosis;

D) vitamin C, Siberian ginseng (Eleuterococcus), ginger

and green tea for increasing resistance to stress, and controlling intestinal problems correlated therewith. The integrator thus obtained is suitable for people who travel widely, live in situations of any kind of stress (family, work, studies and life-stile), managers and for improving physical efficiency and protection against infective diseases.

EXAMPLE 3

Fermented milk prepared with a mixture of lactic bacteria and bifidobacteria.

A 20-30 g sachet of dried concentrated product is thus composed:

S. thermophilus + *L. delbrueckii* substp. *bulgaricus* grown in symbiosis	$50x10^9$ ufc/g
L. acidophilus	$10x10^9$ ufc/g
L. casei	$50x10^9$ ufc/g
L. plantarum	$10x10^9$ ufc/g
Bifidobacteria	$50x10^9$ ufc/g

This composition is inoculated directly into 1000 liters of milk previously pasteurized and cooled to a temperature of 44°C. It is left to incubate at this temperature for the time necessary for the development of the micro-organisms inoculated (7-8 h) and at the end of the process, a fermented milk is obtained with high probiotic qualities, having a pH of 4.1-4.3 and containing

per ml:

1000-2000 x 10^6 ufc	*S. thermophilus*
100-200 x 10^6 ufc	*L. delbrueckii* subsp. *bulgaricus*
10-100 x 10^6 ufc	*L. acidophilus*
10-100 x 10^6 ufc	*L. casei*
10-100 x 10^6 ufc	*L. plantarum*
50-100 x 10^6 ufc	bifidobacteria.

EXAMPLE 4

Food integrators for broilers based on lactic bacteria.

These integrators contain the following lactic bacteria:

L. acidophilus	$10x10^9$ ufc/g
L. plantarum	$50x10^9$ ufc/g
S. thermophilus/L. bulgaricus	$2.5x10^9$ ufc/g
L. salivarius	$1x10^9$ ufc/g

Excipient: lactose

100 g of product are administered to drinking water and this quantity of product is sufficient for 10,000 subjects.

EXAMPLE 5

Feed for broilers based on lactic bacteria as in Example 4 with the addition of exogenous enzymes.

1 g of exogenous enzymes/kg of feed is used.

This mixture allows the hydrolysis of specific glu-

cosides both of the non-starchy type and also those partially indigestible. It develops a synergic effect with the enzymes in the catabolism of polysaccharides of wheat, barley, oats, rye and triticum and contemporaneously integrates the intestinal flora of the animals.

The administration to broilers of both preparations allows an improvement in the retention of nitrogen, growth and ICA; a reduction in cholesterol in the blood and cecum coliforms.

EXAMPLE 6

Food integrator for hens based on lactic bacteria and bifidobacteria.

This integrator contains the following combination of pr micro-organisms;

L. acidophilus	$10x10^9$ ufc/g
L. plantarum	$50x10^9$ ufc/g
L. casei	$5x10^9$ ufc/g
S. thermophilus/L. bulgaricus	$2.5x10^9$ ufc/g
L. salivarius	$1x10^9$ ufc/g
Bifidobacterium bifidum	$50x10^9$ ufc/g

Excipient: lactose

100 g of product are administered to drinking water and this quantity of product is sufficient for 10,000 subjects.

EXAMPLE 7

Feed for hens based on lactic bacteria and bifidobacteria as in Example 3 with the addition of exogenous enzymes.

1 g of exogenous enzymes/kg of feed is used.

Unlike what is specified in Example 5, this mixture contains pr micro-organisms conditioned to the catabolism of polysaccharide monomers and oligomers, in particular arabinose and xylose, particularly studied for developing a synergic effect with the exogenous enzymes in the complete catabolism of polysaccharides of wheat, barley, oats, rye and triticum.

The administration of the preparations of Example 6 and 7 to hens stimulates the animal's appetite and allows an improvement in the phytasic activity in the digestive tract, in the P retention of N and Ca, the ICA laying rate, the egg/hen mass, the specific weight of the eggs and thickness of the shells; a lowering of the pH in the goiter and intestines and cholesterol in the yoke.

EXAMPLE 8

Preparation of an antibiotic-free integrated feed as a substitution of milk destined for calves.

- lactic bacteria:	0.5 %
L. plantarum	$2x10^9$ ufc/g
E. faecium	$2x10^9$ ufc/g
S. thermophilus/L. bulgaricus	$10x10^9$ ufc/g

Excipient: lactose

- dried whey 85 %
- coconut oil 8 %
- fat 4 %
- cereal flour 2 %
- mineral and vitamin integrator 0.5 %

The substitutive integrated feed has a pH equal to 5.0 after re-formation in water at 10%.

EXAMPLE 9

Complementary feed for milk cows/meat cattle based on a mixture of pr micro-organisms.

The complementary feed comprises the following pr micro-organisms:

L. plantarum	$10x10^9$ ufc/g
S. thermophilus/L. bulgaricus	$50x10^9$ ufc/g
Propionibacteria	$10x10^9$ ufc/g
Saccharomyces cerevisiae	$10x10^9$ ufc/g
L. casei	$10x10^9$ ufc/g
L. helveticus	$10x10^9$ ufc/g

Excipients: cereals and their by-products to obtain a charge of $2x10^9$ ufc/g.

30-100 grams/head/day are administered. The preparation increases the ingestion of dry substances, it contributes to reducing post-birth hygiene problems, exerts a detoxifying and hepato-protective action; it increases

the production of milk with a high fat and protein titre; it stimulates rumenal fermentation and favours fibre digestion.

The concentration of live yeasts, total anaerobic bacteria and celluloselytic bacteria in rumenal liquid (ufc log10/ml) gave the following results:

	Control	Preparation (15g/head/day)
- Yeasts	5.40	6.87
- Total anaerobic bacteria	8.98	10.35
- Celluloselytic bacteria	7.28	8.82

EXAMPLE 10

Food integrator for piglets with a mixture of lactic bacteria.

The following ingredients are introduced and mixed in a double-chamber (or V-shaped) mixer having the appropriate capacity:

- Live and vital lactic bacteria in a dried concentrated form ($100x10^9$ ufc/g)

L. acidophilus	$10x10^9$ ufc/g
L. plantarum	$50x10^9$ ufc/g
S. thermophilus/L. bulgaricus	$30x10^9$ ufc/g
E. faecium	$10x10^9$ ufc/g

- A carrier consisting of lactose or whey in powder or any other form, in a quantity sufficient for obtaining a

microbial charge of 2 billion cells per gram of integrator product.

The product thus obtained is introduced, in the desired quantities, into a horizontal mixer and mixed with the products forming the various feeds in the following proportions:

- dried skimmed milk 10%
- dried whey 5%
- soybean flour 10%
- maize flour 20%
- fish meal 5%
- barley flour 5%
- oat flour 2.5%
- barley flakes 20%
- oat flakes 5%
- bran 15%
- mineral salts 2%
- vitamin mixture 1%
- integrators with lactic bacteria 0.5%

WHAT WE CLAIM IS

1. Dietetic and/or pharmaceutical compositions for human and/or animal use based on microbial cultures consisting of autochthonous and allochthonous species, with respect to human beings and animals, selected from the lactic bacterial species *Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei* subsp. *casei, Lactobacillus casei* subsp. *rhamnosus, Lactobacillus zeae, Lactobacillus salivarius, Lactobacillus lactis, Lactobacillus helveticus, Lactobacillus reuteri, Lactobacillus amylovorus, Lactobacillus crispatus, Lactobacillus curvatus, Lactobacillus delbrueckii* subsp. *delbrueckii, Lactobacillus* delbrueckii and all its subspecies, Lactobacillus *gasseri, Lactobacillus johnsonii, Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus,* optionally associated with *Streptococcus thermophilus; Lactobacillus fermentum, Lactobacillus brevis, Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris* and *Leuconostoc* spp.; *Enterococcus faecium, Pediococcus pentosaceus, Pediococcus acidilactici;* Bifidobacteria such as *Bifidobacterium Longum, Bifidobacterium breve, Bifidobacterium bifidum, Bifidobacterium infantis, Bifidobacterium lactis;* and/or propionibacterial species, yeast species and/or mold species; the above species being live and vital and/or devitalized, and said species

being present in microbial cultures in a dried concentrated form with a concentration ranging from 10^6 ufc/g to 10^{11} ufc/g.

2. The compositions according to claim 1, characterized in that they comprise different strains of the same species with a different sensitivity to bacteriophages (lysogeny and lysotypy) and with the same biological and probiotic properties.

3. The compositions according to claim 1, characterized in that they also comprise at lest one of the following components: other micro-organisms, enzymes, mineral salts, vitamins, prebiotics, natural fibres, phyto-derivatives, antioxidants, fermented milk, paps, feeds.

4. The compositions according to claim 1, characterized in that they comprise prebiotics, such as natural fibres.

5. The compositions according to claim 1, characterized in that at least one of the pr micro-organisms is present in a concentration lower than 10^9 ufc/g.

6. The compositions according to claim 1, characterized in that they comprise *Streptococcus thermophilus,* Bifidobacteria such as *Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium lactis, Bifidobacterium bifidum, Lactobacillus acidophilus* in a concentration ranging from 10^9 to 10^{11} ufc/g, *Lactobacillus plantarum, Lactobacillus casei* subsp. *casei, Lactoba-*

cillus delbrueckii subsp. *bulgaricus, Enterococcus faecium* in a concentration ranging from 10^6 to 10^9 ufc/g.

7. The compositions according to claim 1, characterized in that the live and vital and/or devitalized yeasts are yeasts with a low fermentative capacity for probiotic use, rich in essential amino acids.

8. The compositions according to claim 7, characterized in that the yeast is *Saccharomyces cerevisiae* or *Saccharomyces boulardii*.

9. The compositions according to claim 3, characterized in that the natural enzymes consist of a mixture made up of β-glucanase and xylanase produced by micro-organisms of the Thricoderma type.

10. The compositions according to claim 3, characterized in that the natural fibres are selected from fibres of acacia, oats, apples, inulin, psyllium, microcrystalline cellulose.

11. The compositions according to claim 3, characterized in that the antioxidants are natural antioxidants.

12. The compositions according to claim 11, characterized in that the natural antioxidants are selected from oleuropein (from extravirgin olive oil), lycopene (from tomatoes), bioflavonoids (from citrus fruits), phenol components (from red grapes).

13. The compositions according to claim 3, characterized

in that the vitamins and mineral salts are selected from vitamin A, B1, B2, B6, B12, niacin, C, D3, E, folic acid, calcium, phosphorous, magnesium, iron, zinc.

14. The compositions according to claim 3, characterized in that the phyto-derivatives are selected from those extracted from Eleuterococcus and green tea.

15. The compositions according to claim 1, characterized by the presence of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* developed in symbiosis (or proto-cooperation).

16. The use of the compositions according to any of the claims from 1 to 15, as integrators, foodstuffs and/or dietetic-therapeutic products for human and/or animal nutrition.

17. The use of the compositions according to any of the claims from 1 to 15, for preparing integrators, foodstuffs and/or dietetic-therapeutic products, drinks and/or feeds for human and/or animal nutrition.

18. The use according to claim 17, wherein the food products are milk, cheese, paps, homogenized products (based on meat, milk, cheese, fruit, vegetables), dietetic food products destined for diabetics such as jams, chocolate, sweeteners other than sucrose, or animal feeds.

19. The use according to claim 17, wherein the milk is a

fermented or non-fermented milk, with the direct inoculum of pr micro-organisms in a dried concentrated form.

20. The use according to claim 17, wherein the cheese is a therapeutic dietetic cheese obtained by the addition of pr micro-organisms in a dried concentrate form in a certain processing phase of the cheese.

21. The use according to claim 17, wherein the drinks can be instantaneous drinks or water containing the compositions according to any of the claims from 1 to 15.

22. Integrators, foodstuffs, and/or dietetic-therapeutic products, drinks and/or feeds for human and/or animal nutrition characterized in that they contain a composition according to any of the claims from 1 to 15.

INTERNATIONAL SEARCH REPORT

Internat pplication No
PCT/EP 03/01867

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L1/30 A61K35/74 A61K35/72 A23L1/03 C12N1/20
C12N1/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 02 060276 A (MAEYRAE-MAEKINEN ANNIKA ;SUOMALAINEN TARJA (FI); VALIO LTD (FI); V) 8 August 2002 (2002-08-08) page 1, line 10 -page 2, line 19 page 7, line 11 -page 8, line 14 page 10, line 12-32 page 11, line 15-25 page 12, line 6-16 example 1 --- -/--	1,3-6, 16-22

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 06208 A (PIONEER HI BRED INT) 1 April 1993 (1993-04-01) page 3, paragraph 3 page 4, paragraphs 1,5 page 5, paragraph 3 page 6, paragraph 2 example 2	1-3,5,6, 11,12, 14,16, 17,22
X	US 5 716 615 A (CAVALIERE VESELY RENATA MARIA ET AL) 10 February 1998 (1998-02-10) column 1, line 13-16 column 2, line 6-26,30-65 column 3, line 1-12,20-26,41-48	1,3,6, 9-17,21, 22
X	GIROLA M ET AL: "Efficacy of probiotic preparation with living, freeze-dried lactic acid bacteria and yeast on child diarrhoea" BIOSIS, XP002035135 abstract	1,3-17, 22
X	RU 2 178 975 C (BORTS MIKHAIL SAMUILOVICH;NIKOLAEVA ELENA GAVRILOVNA) 10 February 2002 (2002-02-10) abstract	1-3,11, 12,16, 17,22
X	EP 1 177 794 A (LACPRO IND LLC) 6 February 2002 (2002-02-06) paragraphs '0037!,'0043!,'0049! claim 10	1,3,6, 15-19,22
X	GARDINER G ET AL: "EVALUATION OF CHEDDAR CHEESE AS A FOOD CARRIER FOR DELIVERY OF A PROBIOTIC STRAIN TO THE GASTROINTESTINAL TRACT" JOURNAL OF DAIRY SCIENCE, AMERICAN DAIRY SCIENCE ASSOCIATION. CHAMPAIGN, ILLINOIS, US, vol. 82, no. 7, July 1999 (1999-07), pages 1379-1387, XP000850203 ISSN: 0022-0302 page 1380, column 1, paragraph 3 -page 1381, column 2, paragraph 2	1,16-20, 22

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FAMULARO G ET AL: "TRADITIONAL AND HIGH POTENCY PROBIOTIC PREPARATIONS FOR ORAL BACTERIOTHERAPY" BIODRUGS, AUCKLAND, NZ, vol. 12, no. 6, 1999, pages 455-470, XP009001219 ISSN: 1173-8804 page 457, column 2, paragraph 4 page 458, column 1 page 459, column 1, paragraph 4 table II	1,6-8, 10-14, 16,17,22
A	WO 99 62348 A (FITZGERALD GERALD FRANCIS ;STANTON CATHERINE GERARDINE (IE); SULLI) 9 December 1999 (1999-12-09) page 4 page 6	22

INTERNATIONAL SEARCH REPORT

Internati Application No
PCT/EP 03/01867

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 02060276 A	08-08-2002	FI 20010157 A	26-07-2002
		WO 02060276 A1	08-08-2002
WO 9306208 A	01-04-1993	BG 62175 B1	30-04-1999
		BG 98665 A	31-03-1995
		BR 9206505 A	25-04-1995
		CA 2116525 A1	01-04-1993
		CZ 9400595 A3	13-07-1994
		EP 0604543 A1	06-07-1994
		HU 67466 A2	28-04-1995
		JP 6511148 T	15-12-1994
		MX 9205329 A1	01-07-1993
		RO 113477 B	30-07-1998
		RU 2093571 C1	20-10-1997
		SK 32494 A3	10-08-1994
		WO 9306208 A1	01-04-1993
US 5716615 A	10-02-1998	IT 1254210 B	14-09-1995
		DE 69226622 D1	17-09-1998
		DE 69226622 T2	11-02-1999
		DK 555618 T3	17-05-1999
		EP 0555618 A2	18-08-1993
		ES 2120440 T3	01-11-1998
		JP 6056680 A	01-03-1994
RU 2178975 C	10-02-2002	RU 2178975 C1	10-02-2002
EP 1177794 A	06-02-2002	CA 2354331 A1	31-01-2002
		EP 1177794 A2	06-02-2002
WO 9962348 A	09-12-1999	AT 220857 T	15-08-2002
		AU 4161199 A	20-12-1999
		DE 69902260 D1	29-08-2002
		DE 69902260 T2	20-03-2003
		EP 1082021 A1	14-03-2001
		WO 9962348 A1	09-12-1999
		JP 2002516671 T	11-06-2002